

UNCLASSIFIED

AD NUMBER
AD475556
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Oct 1965. Other requests shall be referred to Army Biological Labs., Fort Detrick, Frederick, MD 21701.
AUTHORITY
Biological Defense Research Lab ltr dtd 28 Sep 1971

THIS PAGE IS UNCLASSIFIED

REPRODUCED FROM  
BEST AVAILABLE COPY

AD 475556

TECHNICAL MANUSCRIPT 258

FACTORS AFFECTING RESPONSES  
OF WHITE CARNEAU PIGEONS  
TO RESPIRATORY DOSES OF VEE VIRUS

William S. Miller

DECEMBER 1965

UNITED STATES ARMY  
BIOLOGICAL LABORATORIES  
FORT DETRICK

20050404003

Reproduction of this publication in whole or part is prohibited except with permission of Commanding Officer, U.S. Army Biological Laboratories, ATTN: Technical Releases Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701. However, DDC is authorized to reproduce the publication for United States Government purposes.

DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL LABORATORIES  
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 258

FACTORS AFFECTING RESPONSES OF WHITE CARNEAU PIGEONS  
TO RESPIRATORY DOSES OF VEE VIRUS

William S. Miller

Technical Evaluation Division  
DIRECTORATE OF TECHNICAL SERVICES

Project 1B522301A080

December 1965

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

#### ACKNOWLEDGMENTS

The author wishes to acknowledge the pathology services provided by Captain Michael J. Doherty and Captain Michael E. Seaquist, and the able technical assistance of Mr. Joseph W. Dominik, Mr. John F. Brewer, and Mr. Donald T. Munsey, Jr.

#### ABSTRACT

The ability of Venezuelan equine encephalitis (VEE) virus to induce viremia and elicit formation of serum neutralizing antibodies was studied in White Carneau pigeons. Among birds receiving virus by the respiratory route, an inhaled dose of 3715 MICLD<sub>50</sub> units presented in one minute resulted in both viremic and serologic response in 60 to 80% of the birds. The birds did not respond to a subsequent challenge by the respiratory route, indicating a high level of immunity to VEE virus infection. Birds receiving virus by subcutaneous injection responded identically to the respiratory group in terms of level and duration of viremia and serum neutralizing antibodies. Responses after injection, however, occurred in 100% of the birds; viremias first appeared on day 1 after infection as opposed to viremias first occurring on days 1 or 2 in the respiratory group. Serum neutralizing titers in both groups of birds were followed for 112 days after exposure to or injection of virus. The time of the first appearance of antibodies and the subsequent increase in titers were almost identical for the two routes. Birds showing viremias also had virus in the oral cavity. Because of this factor, and susceptibility to aerosols, it was expected that general bird-to-bird transmission could be demonstrated. That objective, however, was not achieved. Viremic and serologic responses could not be produced unless the dosage rate exceeded the minimum infective dose per minute, regardless of the total dose inhaled.

## I. INTRODUCTION

In a previous study White Carneau pigeons were found to be susceptible to infection by Venezuelan equine encephalitis (VEE) virus by the respiratory route.<sup>1</sup> The minimum infective dose was between 75 and 374 mouse intracerebral LD<sub>50</sub> (MICLD<sub>50</sub>) units inhaled. The criteria of infection were occurrence of viremia and development of serum neutralizing antibodies. The viremias occurred by the second day after exposure and persisted through the third day. Neutralizing antibodies were found in bird sera at the time of sampling three weeks after exposure. The work reported here was conducted to compare response to infection by subcutaneous and respiratory routes. Comparisons were made of the patterns of viremia, excretion of virus, and development of serum neutralizing antibodies. It was also of interest to test for bird-to-bird transmission of the virus, i.e., transmission without an insect vector. As a result of negative data in these latter tests, additional studies were made to evaluate the effect of dosage time on the response to virus received by inhalation.

## II. MATERIALS AND METHODS

The details of techniques were described for the most part in the previous paper.<sup>1</sup> White Carneau pigeons were used that were about six weeks old and had a mean weight of 490 grams. The Trinidad strain of VEE virus was used again as a select egg embryo harvest clarified by centrifugation and treated with antibiotics. The virus had been passed thirteen times through chick embryos. A single lot of virus was employed for all tests with storage between tests at -50 F. Viremias in birds were determined by mouse assay employing eight Swiss-Webster strain mice (8 to 12 grams) per dilution of blood. Serum neutralization (SN) tests were also conducted by mouse assay. The SN index is the difference between viral titers in the presence of normal chicken serum and titers in the presence of test pigeon serum.

Oral and cloacal swabs of potentially infected birds were washed in 5 ml of phosphate-buffered saline (PBS) solution containing 100 units per ml of penicillin and 100 µg per ml of streptomycin. Eight mice were injected by the intracerebral route with appropriate dilutions.

Previous pages were blank, therefore not filmed.

Details of aerosol methods have been described previously.<sup>1</sup> The aerosol particle size distribution at the time of bird exposures had a mass median diameter of about 2 microns. The environmental conditions of the aerosols were controlled to 75 F and 80% relative humidity. Aerosol samples were collected in quadruplicate and assayed separately to provide information on experimental error. Mouse assays were used to estimate aerosol concentrations. In trials where birds were exposed to aerosols for one minute, the heads were inserted through a slit in a rubber diaphragm covering a port in the side of the aerosol vessel. During extended exposures of birds a whole-body procedure was employed with hosts contained in expanded metal cages. In all tests birds were grouped according to test treatment in individual Class III cabinets. The possibility of cross-contamination was thus reduced.<sup>2</sup>

The respiratory volumes of pigeons were based on Guyton's general equation.<sup>3</sup> Because of the extremely consistent body weights of birds a mean breathing rate of 220 ml per minute was applied to calculations of inhaled doses for all hosts.

### III. RESULTS

To determine the protective effect of the original VEE virus infection in birds to subsequent challenge by the respiratory route, five pigeons were exposed to an inhaled dose of 3715 MICLD<sub>50</sub> units over a one-minute period. For viremia determinations, birds were bled daily from the alar vein beginning the 1st day after exposure and ending on the 4th day. Serologic tests were conducted on sera collected before exposure and again 3 weeks later.

A second group of five birds was held in another cabinet system as environmental controls. These birds were bled for viremic and serologic determinations on the same schedule as indicated above.

Two additional control groups were a part of the first exposures. One group of five pigeons was exposed to a low inhaled dose to provide additional verification that the minimum infective dose was between 75 and 374 MICLD<sub>50</sub> inhaled. The actual dose achieved was 51 MICLD<sub>50</sub> units. A second group of five birds was held in the vicinity of the aerosol vessel during the exposure process to test for contaminating virus. Both of these groups were tested for viremia on days 1 through 4 and for serum neutralizing antibodies both before and 3 weeks after the test began. The birds were negative for VEE virus infection by both criteria, as were the environmental controls.



Three weeks following the initial exposures, the group originally receiving 3715 MICLD<sub>50</sub> units inhaled and the original environmental controls were exposed to 3379 MICLD<sub>50</sub> units inhaled, with the dose presented in one minute. Tests for viremia were again undertaken on days 1 through 4 after exposure, and serum neutralization tests were conducted on sera collected three weeks after the challenge. The results are given in Table 1.

Without exception those birds that developed viremia in the first exposures exhibited a significant rise in neutralizing antibodies (>1.0 log). Control birds showed neither viremias nor antibodies. It was also apparent that birds that developed SN titers after the first exposure were resistant to challenge as measured by viremia. Control birds from the first test, however, developed viremia on challenge. The experimental group consisting of exposed birds that did not respond either with viremia or with the production of SN antibodies was not included in the challenge test. Subsequent testing indicated that sub-infective doses resulted in responses upon challenge identical to those of birds with no previous experience with the virus.

A second series of tests compared the responses after exposure to aerosols of VEE virus and after subcutaneous (sc) injection. The response criteria of interest were viremic patterns, occurrence of free virus in oral or cloacal cavities, and development of neutralizing antibody. The eight birds receiving aerosol doses were tested daily for 4 days for viremia and for 10 days by oral and cloacal swabs. The eight birds receiving the sc dose were tested daily for 10 days for viremia and for oral and cloacal virus. Five environmental control birds were examined daily over a 10-day period for viremia and for virus in swab sampler.

The results are presented in Table 2. Some difficulties were experienced in this test with contaminating agent in oral and cloacal swab samples from one control bird. Mouse deaths in the assays occurred in patterns similar to those observed with mice injected with VEE virus. After neutralization tests on numerous samples, however, it was demonstrated that the agent was not VEE virus. Because of this experience, frequent samples of blood and swab washings were subsequently frozen for later confirmation by neutralization tests. All blood samples tested contained VEE virus. The activity of many swab samples, however, was nonspecific. Others, i.e., those with titers of less than two log MICLD<sub>50</sub> units per ml of sample, were too low in viral concentration for reliable neutralization tests or even for reliable passage to increase concentrations.

TABLE 1. RESPONSES OF WHITE CARNEAU PIGEONS TO VEE VIRUS ADMINISTERED BY THE RESPIRATORY ROUTE AND SUBSEQUENTLY CHALLENGED WITH VIRUS BY THE SAME ROUTE<sup>a</sup>

Dose, MICLD <sub>50</sub> with 95% Conf. Limits	Bird No.	Challenge <sub>C</sub> /												
		Viremia, log MICLD <sub>50</sub> per ml blood				SN <sup>b</sup> / Pre- Post- Exp. Exp.		Dose MICLD <sub>50</sub> with 95% Conf. Limits		Viremia, log MICLD <sub>50</sub> per ml blood				SN <sup>b</sup> / Post- Exp.
		Day				1	2	3	4	Day				
		1	2	3	4					1	2	3	4	
3715 in- haled in one minute (2344- 5888)	35	>5.0	>5.0	3.5	<1.5	0.1	2.0	3379 in- haled in one minute (2361- 4781)		<1.5	<1.5	<1.5	<1.5	2.5
	28	<1.5	>5.0	>5.0	<1.5	0.6	2.0			<1.5	<1.5	<1.5	<1.5	1.8
	63	<1.5	>5.0	>5.0	<1.5	0.4	2.9			<1.5	<1.5	<1.5	<1.5	2.5
	40	<1.5	2.7	2.8	<1.5	-0.6	2.2			<1.5	<1.5	<1.5	<1.5	2.9
None (Controls)	45	<1.5	<1.5	<1.5	<1.5	-0.2	0.4	3379 in- haled in one minute (2361- 4781)		3.3	3.1	2.3	<1.5	2.9
	38	<1.5	<1.5	<1.5	<1.5	1.0	0.7			2.9	2.9	2.5	<1.5	2.3
	34	<1.5	<1.5	<1.5	<1.5	-0.5	0.8			1.8	3.2	2.6	<1.5	2.2
	50	<1.5	<1.5	<1.5	<1.5	0.4	0.1			<1.5	3.5	2.5	<1.5	1.9
	62	<1.5	<1.5	<1.5	<1.5	0.5	0.1			<1.5	<1.5	2.4	3.0	1.1

a. Aerosol Conditions: 80% relative humidity at 80 F.

b. Log units of virus neutralized.

c. Birds were challenged 21 days after the original exposure.

TABLE 2. RESPONSES OF WHITE CARNEAU PIGEONS TO VEE VIRUS BY THE RESPIRATORY<sup>2</sup>/ AND SUBCUTANEOUS ROUTES

Dose, MICLD <sub>50</sub> with 95% Conf. Limits	Bird No.	Viremia, Log MICLD <sub>50</sub> per ml of blood										Positive Oral Swabs
		Day After Dosage										
		1	2	3	4	5	6	7	8	9	10	
1349 inhaled in one minute (501-3548)	89	>3.5	>3.5	>3.5	<1.5							+
	92	>3.5	>3.5	>3.5	<1.5							
	21	>3.5	>3.5	>3.5	<1.5							
	9	<1.5	3.5	2.8	>3.5							
	14	<1.5	2.8	>3.5	2.4							
	85	<1.5	<1.5	<1.5	1.7							
	74	<1.5	<1.5	<1.5	<1.5							
	7	<1.5	<1.5	<1.5	<1.5							
506 by Subcutan- eous injec- tion (214-1202)	23	2.7	3.4	3.2	3.2	3.0	1.9	1.9	<1.5	<1.5	1.5	+
	79	>3.5	4.0	4.0	1.7	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	
	90	2.0	>3.5	3.5	2.5	<1.5	<1.5	1.8	1.8	1.8	<1.5	
	1	3.2	>3.5	3.5	1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	
	5	>3.5	>3.5	2.1	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	
	8	3.0	>3.5	1.7	<1.5	Died						
	13	2.6	<1.5	2.6	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	
Five Environ- mental Controls	20	4.5	1.7	1.8	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	
		<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	

a. Aerosol Conditions: 80% relative humidity at 80 F.

3. Aerosol Conditions: 80% relative humidity at 80 F.

In Table 2 the viremic titers may be considered specific except those of 2.0 log units or less, which were not verified.

On the basis, then, of titers occurring primarily to day 5 there was a marked similarity of viremic level and duration between the two routes. There was a marked difference, however, in the efficiency of viremic infection for the two routes. By subcutaneous injection every bird was viremic on the 1st day after inoculation, but hosts receiving doses by the respiratory route were not all infected. Lack of complete response in all birds has been characteristic of exposure to aerosols at concentrations as high as 4000 MICLD<sub>50</sub> units inhaled.

VEE virus was recovered and identified from two oral swabs. One swab was made of the oral cavity of bird 82 (respiratory group) on day 3 after exposure. The second swab recovery, with viral identification, was from bird 23 (injected group) on day 5 after inoculation. In addition to the blood and swab samples indicated above, samples were also collected on days 42 and 43 and again on days 80 and 81. There was no evidence of infective agent in any of the later samples.

SN indices were determined for sera collected from all birds 0, 5, 10, 20, 40, 80, and 112 days after exposure or injection. Indices are plotted in Figure 1 for each route for viremic hosts, for exposed but non-viremic birds, and for environmental controls, which showed no sign of viremic infection. The lack of significant neutralizing antibody formation in the case of the non-viremic birds is consistent with earlier results regarding the correlation between viremia and formation of neutralizing antibody.

The similarity of serologic response to virus presented by the respiratory route and by sc injection is apparent.

Based on the evidence of viral infection by respiratory exposure and the presence of virus in the oral cavity, the possibility of cross infection among birds was investigated. In the earlier test,<sup>1</sup> normal birds housed with infected birds did not exhibit viremia or neutralizing antibodies. It was proposed that the holding areas were too large for intimate contact. A system was devised to give maximum contact between infected and normal birds by the respiratory route. The apparatus employed is illustrated in Figure 2. Six birds exposed to a respiratory dose of 7447 MICLD<sub>50</sub> units inhaled were placed on one box and six normals were placed in the second. All air for the normal birds was received through the connecting duct from the infected birds. The air flow was about 12 liters per minute. To insure that arthropods would not pass from infected to normal birds, a screen of 0.25 mm pore size was placed across one mouth of the duct.

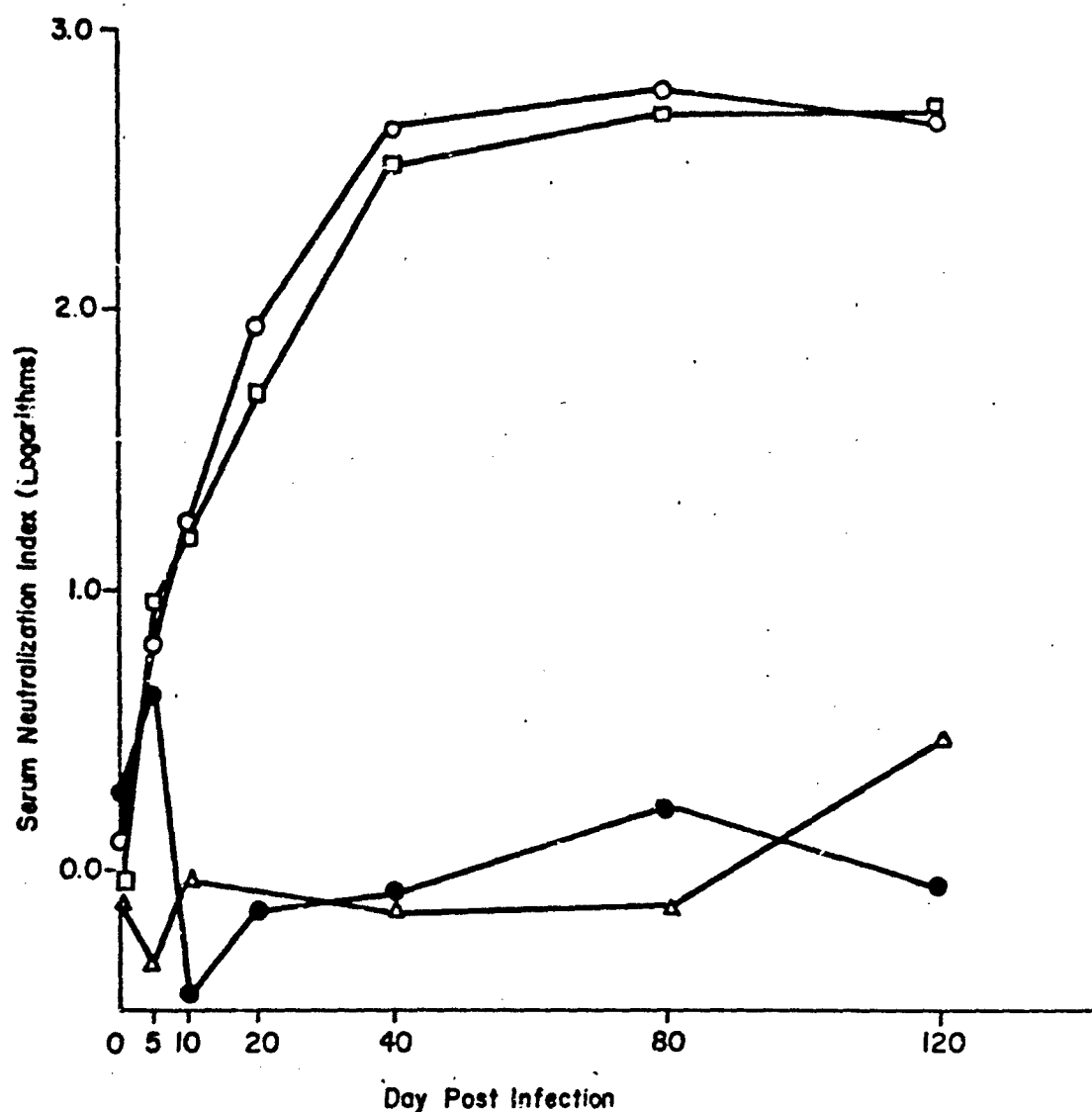


Figure 1. Results of Neutralization Tests Against Venezuelan Equine Encephalitis Virus by Sera Collected from White Carneau Pigeons at Various Periods after Injection. Symbols: O = birds that developed viremia following respiratory dosage; Δ = birds that did not develop viremia following respiratory dosage; □ = birds that developed viremia following dosage by the subcutaneous route; ● = birds that served as environmental controls and were not viremic.

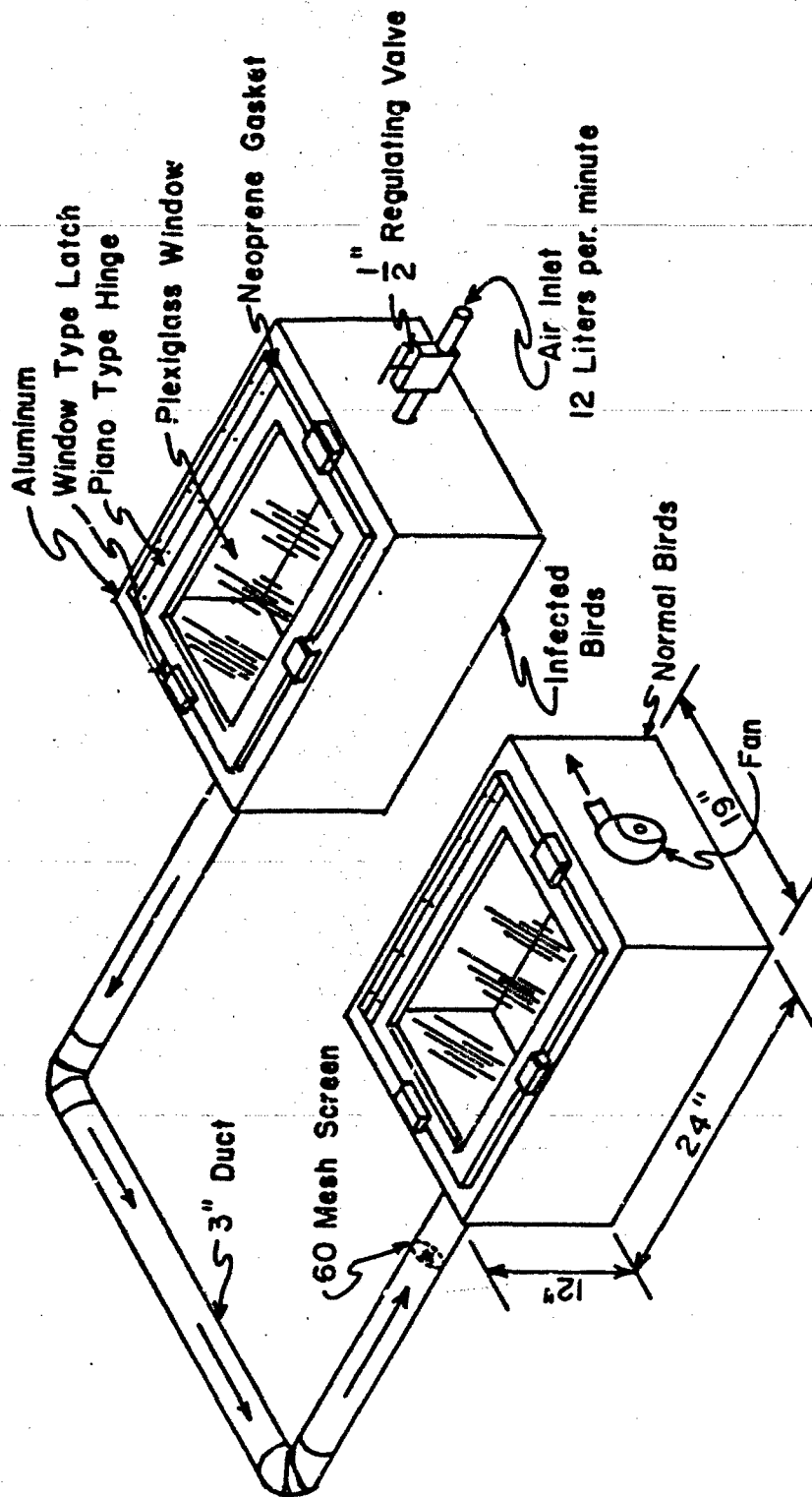


Figure 2. Apparatus for Study of Bird-to-Bird Infection with Venezuelan Equine Encephalitis Virus by the Respiratory Route. Six infected birds were placed in one box and six normals in the second. The boxes were placed in separate safety cabinet systems to permit periodic bird removal for bleedings without allowing contact.

After initiation of the test the birds remained in their respective boxes for three weeks except for a short period, daily, for the first ten days, during which birds were bled for viremia determinations and the usual oral and cloacal swab samples were collected. Because the holding boxes were located in separate enclosures there was no chance of virus transmission except by way of the connecting duct. The results obtained in the cross-infection test are presented in Table 3.

In the case of exposed birds a number of blood samples yielding higher titers were tested for specificity by the neutralization test. All samples contained VEE virus except in one instance, and in that case a rise in serum neutralizing antibody was found at 20 days after exposure. The blood sample from bird 55 was confirmed for VEE virus specificity on day 7. The high level of viremia continued for an unusually long period until the bird died of a bacterial pneumonia on day 10.

Among the oral and cloacal swab samples collected from exposed birds, 37 showed lethality for assay mice within the normal incubation period for VEE virus. The concentrations ranged from two to three log MICLD<sub>50</sub> units per swab. Thirteen samples gave titers sufficiently high to warrant tests for specificity. The original samples were injected into mice. Brains were harvested from those that died during the incubation period, homogenized in beef heart infusion broth, and centrifuged at low speed. The supernatants were then diluted 1:1 with inactivated normal or immune chicken serum, incubated at 37 C for one hour, and refrigerated at 4 C for two hours. Samples at appropriate dilutions were injected into mice.

Of the 13 samples only two contained detectable VEE virus. The samples were both from bird 55; both were oral swabs, and were originally collected on days 6 and 10.

Among the normal birds, a significant viremia was found in one bird only (number 63). Two blood samples from that host, collected on days 9 and 10, were tested for specificity and both were positive. Among the 120 swab samples collected from the normal birds, 17 cloacal swabs occurring at random yielded titers of unknown agent of two to three log MICLD<sub>50</sub> units per swab and one oral swab indicated a titer of two log MICLD<sub>50</sub> units. A number of these swab samples showing higher titers were injected into mice after a freeze-thaw cycle, but no responses were obtained. The labile nature of the agent suggested a non-viral organism.

TABLE 3. TESTS FOR CROSS-INFECTION BETWEEN INFECTED AND NORMAL WHITE CARNEAU PIGEONS BY THE RESPIRATORY ROUTE<sup>a</sup>

Dose, MICLD <sub>50</sub> with 95% Conf. Limits	Bird No.	Viremia, log MICLD <sub>50</sub> per ml blood									
		Test Day									
		1	2	3	4	5	6	7	8	9	10
7447 inhaled in one minute (5623-9772)	51	>3.5	>3.5	1.7	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	52	>3.5	>3.5	2.9	<1.5	<1.5	<1.5	1.8	1.3	1.7	1.9
	54	>3.5	3.2	3.3	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	57	>3.5	3.2	3.3	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	56	<1.5	>3.5	3.2	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	2.2
	55	<1.5	1.4	<1.5	2.7	2.3	>3.5	>3.5	>3.5	>3.5	>3.5
None	63	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	1.8	1.7	2.9	3.8
	59	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	60	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	1.8	<1.5
	58	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	61	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5
	62	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5

<sup>a</sup>. Aerosol Conditions: 80% relative humidity at 80 F.



Surviving birds were tested for neutralizing antibodies on day 20. All of the exposed birds tested showed increases in indices of greater than one log. None of the normal birds showed a rise in SN index. All were tested except bird 63, which died on the 19th test day of unknown causes. It was concluded that only one bird out of six (number 63) was infected by the abnormal contact condition.

It was apparent from these data as well as from earlier tests with pigeons that bird-to-bird infections were unlikely, even in view of the dose-response relationship by the respiratory route and evidence of oral virus. One possibility explaining these findings lay in a difference in dosage. In exposures to controlled viral aerosols, the dose was given in a one-minute period. Exposures of birds to virus shed from infected hosts on the other hand may have been a case of dosage at a lower rate, although the accumulated total dose was relatively high.

A study was designed to permit exposure of pigeons to controlled aerosols, with doses per minute less than the minimum infective dose but with the total inhaled dose far greater than that level. The method of achieving these conditions is illustrated by the plot in Figure 3. An aerosol of VEE virus was produced with a mass median diameter of about 4 microns. Following collection of a control aerosol sample at a midpoint of 4 minutes after dissemination the aerosol purge system was operated for 20 minutes to reduce the viral concentration. After the physical reduction of viral units, aerosol samples were collected in quadruplicate. At this time and subsequently, the mass median diameter of the aerosol was 2.0 microns. The cloud was then allowed to undergo natural decay for 45 minutes, at which time another set of samples was collected. This was the point at which the cloud was expected to approach non-detectable levels. Further natural decay proceeded for a period calculated to yield the desired dosage level. Birds were then exposed for 60 minutes. Other tests had indicated that infectivity properties for guinea pigs remained constant over the cloud age of interest. Also, evidence from numerous other tests was available to indicate that decay of the aerosol was log linear over the period of interest. Thus the two cloud concentration estimates obtained from the samples collected after the physical reduction could be employed to obtain, by extrapolation, actual estimates of aerosol levels during the extended bird exposures. Total doses were estimated by integration of that segment of the concentration time curve pertaining to the bird exposure period. The definite integral employed was:

$$d = \int_{t_1}^{t_2} I C_0 e^{-kt}$$

where:  $d$  = dose in MICLD<sub>50</sub> units

$t$  = time in minutes

$I$  = respiratory volume per minute in milliliters

$C_0$  = concentration of aerosol at beginning of exposure period in MICLD<sub>50</sub> units per liter of air in one minute

$k$  = decay constant

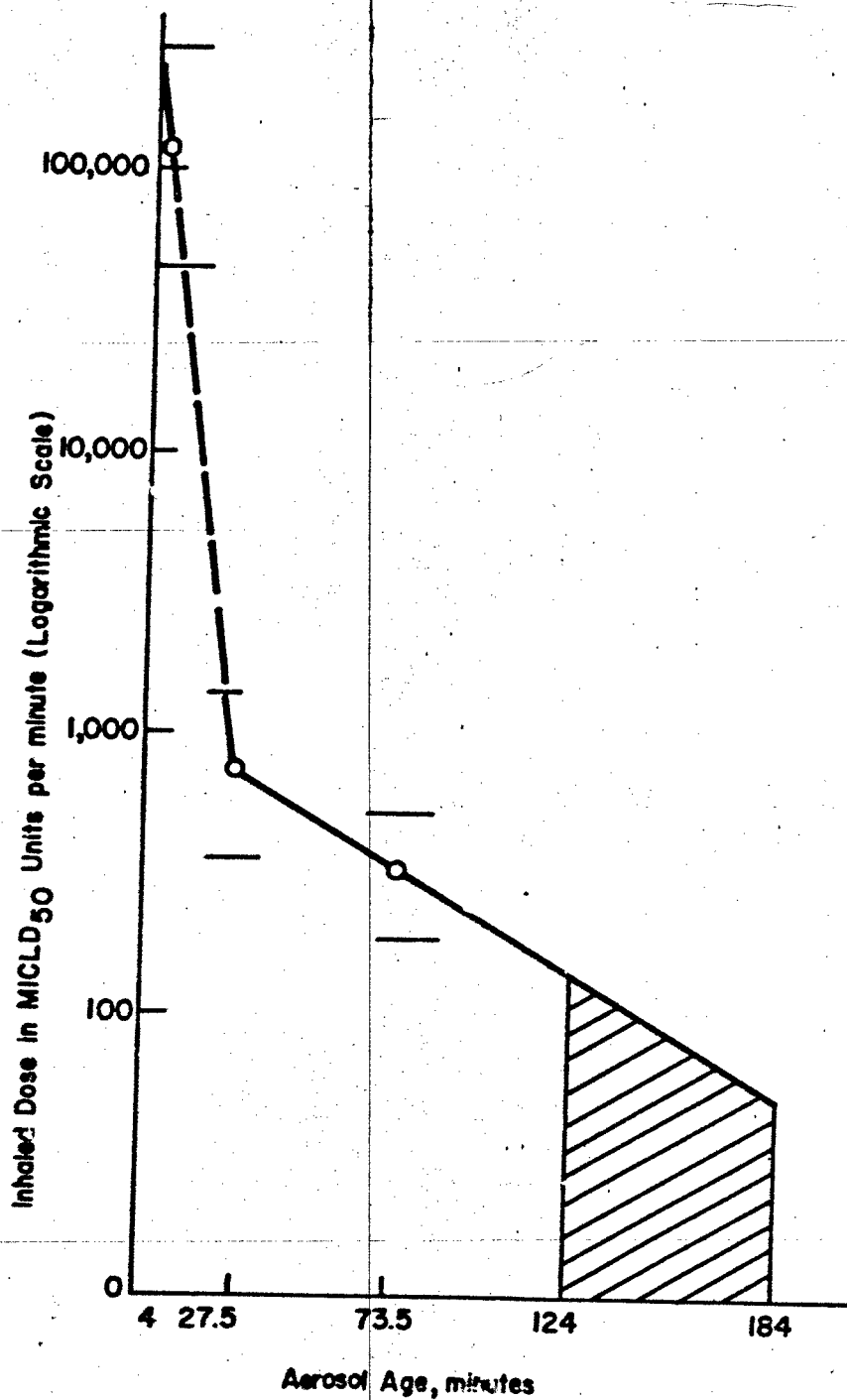


Figure 3. Aerosol Concentration of VEE Virus as a Function of Age. Concentrations are in terms of doses per minute for White Carneau Pigeons expressed as MICLD<sub>50</sub> units. The open circles indicate aerosol concentrations estimated by sampling. The solid line indicates the periods of natural cloud total decay; the dotted line indicates a period of mechanical purging. The hatched area illustrates the dosage inhaled by pigeons during a 60-minute exposure. Bands above and below the open circles indicate 95% confidence limits.

In later tests it became clear that if the dose in the first minute exceeded the minimum infective dose for one minute, a normal viremic and serologic response followed. This occurred, for example, in the first test because of incorrect prediction of cloud characteristics, with a resulting first-minute dose of 304 MICLD<sub>50</sub> units inhaled. In that test, five of seven birds responded with viremias at levels and for durations identical to those found previously for aerosol-exposed hosts. Each of the viremic birds showed significant levels of serum neutralizing antibodies 3 weeks later and virus was identified as VEE in at least one oral swab collected on day 3 after exposure. These observations supported the idea that the response was a function of exposure rate.

The next effort resulted in more accurate predictions. The actual cloud concentration and decay properties were not greatly different from test design calculations. Eight pigeons were exposed to a total inhaled dose of 4544 MICLD<sub>50</sub> units over a 60-minute period. The dose in the first minute, which was the highest one-minute dose during the entire exposure (Fig. 3), was 124 MICLD<sub>50</sub> units inhaled. Birds were tested for viremia from day 1 through day 10 after exposure and daily swab samples of the oral cavity and of the cloaca were obtained. In addition, birds were bled at 21 days for SN tests. The results were completely negative. VEE virus did not occur in the blood or in swab samples. Furthermore, SN titers at 21 days did not exceed 0.7 log unit. Four of these birds were subsequently challenged with VEE virus by the respiratory route. Three of four showed normal viremias, three gave oral swab isolates that were confirmed, and SN indices three weeks later were 2.0 log units or higher. The fourth bird did not respond in terms of either viremia or SN titer.

One additional test, to be reported later, resulted in conclusions similar to those above. Exposure of 15 pigeons to a total inhaled dose of about 2000 MICLD<sub>50</sub> units with a first-minute dose of 22 MICLD<sub>50</sub> units inhaled resulted in viremias in only two hosts.

Many birds sacrificed at the completion of individual experiments and the relatively small number that died during tests were submitted to a pathology laboratory. There was no evidence on the basis of histopathologic studies that the VEE virus infections were characterized by focal encephalitis. One instance of a meningitis was reported and in several cases trichomonad infections were found. The only consistent disease pattern was noted among the birds confined in the small boxes described previously in the cross-infection study. It was reported that all birds, upon sacrifice, showed necrotizing bronchitis and pneumonia with abscess formation. The one bird (number 55) that died 10 days after exposure exhibited a bacterial pneumonia. This bird also exhibited an abnormally late and long-term viremia due to VEE virus. It appeared in this case that the secondary infection may have interrupted the viral clearance mechanism in an otherwise resistant bird. The bias introduced into the cross-infection test must be recognized. It is possible, then, that none of the normal birds would have been infected had the exposed mates showed a normal viremia.

### III. DISCUSSION

These studies were conducted to understand better the parasite-host relationship of VEE virus and White Carneau pigeons. Inhalation of virus in small particles by normal birds results in infections when the inhaled dose in one minute is more than 75 MICLD<sub>50</sub> units. The infection is characterized by viremia of 2 or 3 days' duration and the development of serum neutralizing antibodies.<sup>1</sup> The presence of these antibodies was clearly associated with protection against aerosol challenge.

Respiratory resistance factors were strongly suggested by the general failure of normal birds to be infected by exhaust air from infected birds excreting virus by way of the oral cavity. Additional evidence of such nonspecific resistance was suggested by the immediate occurrence of viremias in all birds injected with VEE virus although only a partial response with slightly delayed onset was routinely obtained in birds receiving an inhaled dose as much as seven times higher. However, this comparison of viremic responses to inhaled and injected doses is confounded by unknown retention of the former. However, the difference in response even in the presence of a markedly higher aerosol dose argues against an assumption of respiratory tract resistance factors.

The magnitude of the resistance factors in immunological virgins was shown by the resistance to comparatively much higher doses presented over extended periods of time. It was obvious that the critical factor in the induction of viremia and stimulation of neutralizing antibody formation was a dosage rate greater than the rate of nonspecific neutralization of agent.

The nature of the nonspecific resistance to VEE virus is of course of interest. Possible mechanisms could include physical removal, action of the phagocytic system, properdin-type antiviral substances, or autointerference. The latter possibility is suggested by the nature of the dosage scheme employed. Since graded doses were achieved by natural decay of the aerosol, the ratio of dead to live virus necessarily increased as dosage level decreased. Thus, conditions were favorable for autointerference as the cause of the dose response patterns, including those associated with extended exposures. Additional study is required to evaluate this possibility.

The agent neutralization indicated above was very effective in preventing formation of SN protective antibodies. It is of further interest to determine the dose-response relationship subsequent to the extended exposure experience for detection of any change in response of the birds to VEE virus. Such tests are in progress. Attempts have been made to detect hypersensitivity in exposed birds but without success. Skin tests have been routinely negative. In addition, anaphylactic shock has not been demonstrated.

A statement cannot be made that bird-to-bird transmission does not occur in nature. The data reported here only imply that dosage must exceed the minimum infective dose per minute in order to infect. Wild birds may in fact be far more susceptible to small doses than are pigeons. In this event excreted virus may be at sufficient peak concentration to produce a normal viremic infection and thus contribute to either mosquito-bird cycles or to bird-to-bird cycles.

LITERATURE CITED

1. Miller, W.S. April 1965. Susceptibility of White Carneau pigeons to respiratory infection by Venezuelan equine encephalitis virus, (Technical Manuscript 213). Technical Evaluation Division, U.S. Army Biological Laboratories, Frederick, Maryland. (Also to be published in J. Epidemiol. 1966.)
2. Jemski, J.V.; Phillips, G.B. 1965. Aerosol challenge of animals, p. 273-341. In W.T. Gay (ed.) Methods of animal experimentation, Vol. 1. Academic Press, Inc., New York.
3. Guyton, A.C. 1947. Measurement of the respiratory volumes of laboratory animals. Amer. J. Physiol. 150:70-77.

Previous pages were blank, therefore not filmed.

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D		
(Security classification of title, body of abstract and indexation annotation must be entered when the overall report is classified)		
1 ORIGINATING ACTIVITY (Corporate author)		2a REPORT SECURITY CLASSIFICATION
U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701		Unclassified
		2b GROUP
3 REPORT TITLE		
FACTORS AFFECTING RESPONSES OF WHITE CARNEAU PIGEONS TO RESPIRATORY DOSES OF VEE VIRUS		
4 DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5 AUTHOR(S) (Last name, first name, initial)		
Miller, William S.		
6 REPORT DATE	7a TOTAL NO OF PAGES	7b NO OF REFS
December 1965	24	3
8a CONTRACT OR GRANT NO.		8b ORIGINATOR'S REPORT NUMBER(S)
A. PROJECT NO. 1B522301A080		Technical Manuscript 258
c.		9a OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.		
10 AVAILABILITY / LIMITATION NOTICES		
Qualified requestors may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.		
11 SUPPLEMENTARY NOTES		12 SPONSORING MILITARY ACTIVITY
		U.S. Army Biological Laboratories Fort Detrick, Frederick, Maryland, 21701
13 ABSTRACT		
<p>The ability of Venezuelan equine encephalitis (VEE) virus to induce viremia and elicit formation of serum neutralizing antibodies was studied in White Carneau pigeons. Among birds receiving virus by the respiratory route, an inhaled dose of 3715 MICLD<sub>50</sub> units presented in one minute resulted in both viremic and serologic response in 60 to 80% of the birds. The birds did not respond to a subsequent challenge by the respiratory route, indicating a high level of immunity to VEE virus infection. Birds receiving virus by subcutaneous injection responded identically to the respiratory group in terms of level and duration of viremia and serum neutralizing antibodies. Responses after injection, however, occurred in 100% of the birds; viremias first appeared on day 1 after infection as opposed to viremias first occurring on days 1 or 2 in the respiratory group. Serum neutralizing titers in both groups of birds were followed for 112 days after exposure to or injection of virus. The time of the first appearance of antibodies and the subsequent increase in titers were almost identical for the two routes. Birds showing viremias also had virus in the oral cavity. Because of this factor, and susceptibility to aerosols, it was expected that general bird-to-bird transmission could be demonstrated. That objective, however, was not achieved. Viremic and serologic responses could not be produced unless the dosage rate exceeded the minimum infective dose per minute, regardless of the total dose inhaled.</p>		

DD FORM 1473

Unclassified

Security Classification